Figure 1A Nucleotide sequence of inserted environmental DNA (mHKcel cellulase)

ATCTTTAGAG	ATGAGCGTGA	TAGTCAGTGG	ACCTTAAAAG	AACTTAAAAG	50
			CCATCTCTCT		100
ATGGGGGATT		ACACAACGAT		GTGTGTGATT	150
TATTATGAAC	AAAATGACAC	GTCTTACCGG	GTGAGAAAAA	ACGCTACCGT	200
GGCGTCATTG	ACATCGAATA	ACCAAGCAGA	ATATGCCGCT	TTGCATTTAG	250
	AACTTGAAGG			TATCACCATT	300
TACGGTGATT	CTCAAGTTGT	GATCAATCAG	TTAAAAGGAG	AATGGGCCGT	350
GTATGGAGGA	GGTGTTAAAT	AAATGGGCTG	ACCCGTATTG	ATCAAGCATT	400
TAGCTAAATT	AGGCATGACC	GCTACTTATA	AGTTAATCCC	CCGTAAAGAA	450
AACCGCGAAG	CCGATCAACT	GGCTACACAA	GCGTTAAACG	GGCAAGAAAT	500
TATAAGTCAA	CGTGATATCA	GTGAGCGTGG	TGCAGATTAG	GCTGCACCGC	550
GCAAAAAAAG	TCAACGTGTT	TAGGAATGGA	CAGGGATTAA	AGCAACATAA	600
TTCTCTCTAA	GCAAACGTTG	CGACAGCAAG	AGAGAAGCAT	ATAAGGTTTT	650
TCTGAGTTAG	TCTATTTATA	CCAATGTCCA	CGTACTAAAT	AAACCTCTCA	700
TCAAAGTGGA	TTTTTTGATT	AATTCACTTC	CACTCCTACC	TTTATCTATA	750
TAAATTAGTT	CCTTTTTTGT	TAATAATCAC	TAATTTTGGC	GGTATTTTTT	800
AATAGAAATA	TATGCTAGAT	TATAAACTAG	TAGCCGTATA	GAAGGTGGTG	850
ATTGCCCCTA	TAAGAGACGT	CTGGCAAACA	TAAAAGCATC	GCATTATTAT	900
AATCGAAAGG	TGGAGATGAG	ACATGGGTTA	TACCCAAGCT	AAGTGTATGG	950
TGAAAAAAAC	GGTCTTGTTT	GGTTTAATTC	TCTGTTTAGG	TGTGTCAATG	1000
TTTGTACCAG	TTACATCAGC	TGAAGATAGG	GTCTCTTCGT	CACAGGTGGA	1050
TATCCAATCA	TATGTAGCAG	ATATGCAACC	TGGCTGGAAT	TTAGGTAATA	1100
CATTTGATGC	GATAGGAGAT	GATGAAACAG	CATGGGGAAA	CCCTCGTGTA	1150
ACGAGAGAAT	TAATAGAAAT	GATTGCTGAT	GAAGGGTATA	AAAGTATTCG	1200
TATCCCAGTC	ACATGGCAAA	ATCAAATGGG	TGGTTCTCCA	GATTATACAA	1250
TTAATGAAGA	TTATATCAAG	CGGGTAGAGC	AAGTGATAGA	TTGGGCGTTG	1300
GAGGAAGACT	TGTATGTGAT	GTTAAATGTG	CATCATGACT	CATGGCTGTG	1350
GATGTATGAT	ATGGAACATA	ACTATGATGA	GGTGATGGCA	AGATATACAG	1400
CTATTTGGGA	ACAATTGTCG	GAAAAATTCA	AAAACCACTC	CCATAAGTTG	1450
ATGTTTGAGA	GTGTCAATGA	GCCTAGGTTT	ACGCAGGAGT	GGGGAGAGAT	1500
TCAAGAAAAT	CATCATGCTT	ACTTAGAAGA	TTTAAATAAG	ACGTTCTATT	1550
	AGAGTCAGGA		TGGAGCGCCC	TTTAGTATTG	1600
	AAACAGCCAC		TTACTAGATC		1650
AACAATGGAA	GACTTGGATG	ACCCTCATTT	AATTGCCACG	GTTCATTATT	1700
ATGGCTTTTG	GCCCTTTAGT		CAGGGTACAC		1750
	AACAAGATAT		TTTGACCGTG		1800
			AGGTGAATTT		1850
			AAGGTGAGAA		1900
			CGTGATATAA		1950
			AACTTATTCA		2000
			GGGAGGGGCG		2050
			GGAGAGCCAA		2100
			GCTAACTGCC		2150
			ATGAGCTAGC		2200
			TTAATTACCC		2250
			TAATTCTGGA		2300
			CAGTCGAAAA		2350
TCAATATGGC	ATTTTGCGAT	CCCTACCCAT	TTTAATGGTG	ATAGTCTTGC	2400

Figure 1B

				~~~~~~~~	0450
	GCTGTTTATG			CCGCAAGATT	2450
GGACGTCATT	TAAAGAATTT	GGCGAGGCGT	TTTCCCCTAA	TTACGCCACA	2500
GGGGAAATTA	TTATAACAGA	AGCCTTCTTT	AACGCGGTAC	GGGATGATGA	2550
TATCCATTTA	ACATTTCATT	ATTGGAGCGG	AGAGACGGTG	GAATATACAT	2600
TACGTAAAAA	TGGAAATTAT	GTTCAAGGTA	GACGGTAACA	TGATTTTAAT	2650
TAATAGATAA	AACAGCCTAC	CTATCGTTTT	TGGAAGAAGG	CAAACGAATC	2700
TCATCTTACC	AACACCGTGC	TTTAGAACTT	TAGAAGTGAC	GGTGTTTTTT	2750
AAGACATGAG	GAGAGACAAT	CCTCTATCAA	CAGTCACCAA	TTTTTATTCA	2800
GGAGGTGTCA	AGTTATCTAA	CGTTCTATGA	ATGCATATAG	TTTCTGACGA	2850
ATAAACATAG	TTAAAAAGAA	GTGAGCCTAG	TTCCCGAGGG	GAAGGGGATA	2900
ATGCCAACGT	ATTGGATTAA	AGTACCTTCT	TGATAAAAAG	AAAGGGTTTT	2950
CAAGAGGTGG	AAATGGGCTC	GTTTGTTATA	CTTTAATTAC	ACCTTGGAAC	3000
GTCATTTTGG	CGGTGCTACT	TAGTAAGATG	ACTGACATCA	TAAAAGAGGA	3050
GTGGGTTCGA	TGGCTTTAAT	TCAATTAAGC	TTTAAATCAC	GAGCATTAAT	3100
GTTGCAAACC	TCTGTCAATG	TTTTATTACC	GGTGGGAATG	AATGCGGTAG	3150
ATTTTACACC	AAGTGATGAT	TTTTCTTATG	TTACTGACCC	TTTTCCTGTC	3200
CTATATCTTT	TGCATGGTGC	AACTGATGAT	TATTCAGCAT	GGCTACGTCT	3250
GTCCTCCATT	GAACGATATG	CTGAAGAAAA	AAAATTGGCG	GTCGTCATGC	3300
CAAATGCTGA	TATGAGTGCG	TATACGGATA	TGGTACATGG	ACATCGTTAC	3350
TGGACGTATA	TTAGTAAGGA	GCTGCCTGAG	TTTATCAAAG	CGACTTTTCC	3400
TATTTCTCAG	CACCGTGAAG	ACACCTTTGC	GGCTGGTCTG	TCTATGGGAG	3450
GATACGGGGC	TTTTAAATTA	GCGTTGCGGC	AACCGGAACG	CTTCGCTGCA	3500
GNTGTGTCAT	TATCAGGTGC	AGTTGATATG	AGAGAAGCAA	GTCAACCAGA	3550
CTCCCTATTT	GTGAACGCAT	TTGGTGAAGG	GACGAAAATC	GCAGGGACAG	3600
ATCTTGATCT	TTTTCATTTA	ATTAAAAAAGT	TGGGGGTATA	TGAAGGGGCT	3650
AAACCAGCCC	TTTTTCAAGC	GTGTGGGACA	GAGGACTTTT	TATATGAAGA	3700
TAATGTGAGA	TTTAGAGATT	ATGCACGACA	AGTGAATGCC	GATTTAACTT	3750
ATGAAGAAGG	TCCTGGTGGT	CATGAATGGG	CTTATTGGGA	TAGAAT	3796

Figure 2 ORF Nucleotide sequence of mHKcel cellulase gene

ATGGGTTATA	CCCAAGCTAA	GTGTATGGTG	AAAAAAACGG	TCTTGTTTGG		50
TTTAATTCTC	TGTTTAGGTG	TGTCAATGTT	TGTACCAGTT	ACATCAGCTG		100
AAGATAGGGT	CTCTTCGTCA	CAGGTGGATA	TCCAATCATA	TGTAGCAGAT		150
ATGCAACCTG	GCTGGAATTT	AGGTAATACA	TTTGATGCGA	TAGGAGATGA		200
TGAAACAGCA	TGGGGAAACC	CTCGTGTAAC	GAGAGAATTA	ATAGAAATGA		250
TTGCTGATGA	AGGGTATAAA	AGTATTCGTA	TCCCAGTCAC	ATGGCAAAAT		300
CAAATGGGTG	GTTCTCCAGA	TTATACAATT	AATGAAGATT	ATATCAAGCG		350
GGTAGAGCAA	GTGATAGATT	GGGCGTTGGA	GGAAGACTTG	TATGTGATGT		400
TAAATGTGCA	TCATGACTCA	TGGCTGTGGA	TGTATGATAT	GGAACATAAC		450
TATGATGAGG		ATATACAGCT				500
		ATAAGTTGAT				550
CTAGGTTTAC		GGAGAGATTC				600
TTAGAAGATT	TAAATAAGAC	GTTCTATTAT	ATTGTCAGAG	AGTCAGGAGG	•	650
CAATAATGTG	GAGCGCCCTT	TAGTATTGCC	TACGATAGAA	ACAGCCACGT		700
	ACTAGATCGC		CAATGGAAGA			750
		TCATTATTAT				800
		GTTTTGAACA				850
TAGACACGTT	TGACCGTGTT	CATAACACAT	TTACAGCGAA	TGGGATCCCA		900
GTTGTATTAG	GTGAATTTGG		TTTGATAAAA			950
CATTCAGCAA	GGTGAGAAAT	TAAAATTTTT	TGAGTTTCTC	ATCCATCATC		1000
TCAATGAACG	TGATATAACC		GGGATAACGG			1050
	CTTATTCATG		GAATTTCATG			1100
		CTGCTACAGC				1150
		AGAGATCAAC				1200
GGAAATGAGC		ACAGGCAGGG				1250
			AACGCTAAAA			1300
		GGCCAATTAG				1350
		AGACTGGCGT				1400
CGTGCCAACA		CAGATGGCTC				1450
CTACCCATTT		AGTCTTGCGA				1500
AACGGAGAAT		GCAAGATTGG				1550
CGAGGCGTTT		ACGCCACAGG				1600
		GATGATGATA				1650
		ATATACATTA	CGTAAAAATG	GAAATTATGT		1700
TCAAGGTAGA	CGGTAA					1715

Figure 3 Amino acid sequence of cellulase mHKcel

MGYTQAKCMV KKTVLFGLIL CLGVSMFVPV TSAEDRVSSS QVDIQSYVAD	50
MQPGWNLGNT FDAIGDDETA WGNPRVTREL IEMIADEGYK SIRIPVTWQN	100
QMGGSPDYTI NEDYIKRVEQ VIDWALEEDL YVMLNVHHDS WLWMYDMEHN	150
YDEVMARYTA IWEQLSEKFK NHSHKLMFES VNEPRFTQEW GEIQENHHAY	200
LEDLNKTFYY IVRESGGNNV ERPLVLPTIE TATSQDLLDR LYQTMEDLDD	250
PHLIATVHYY GFWPFSVNIA GYTRFEQETQ QDIIDTFDRV HNTFTANGIP	300
VVLGEFGLLG FDKSTDVIQQ GEKLKFFEFL IHHLNERDIT HMLWDNGQHL	350
KRETYSWYDQ EFHDILKASW EGRSATAESN FIHVKDGEPI RDQHIQLYLN	400
GNELTALQAG DESLVLGEDY ELAGDVLTLK AGILTRLITP GQLGTNAVIT	450
AQFNSGADWR FQLQNVDVPT VENTDGSIWH FAIPTHFNGD SLATMEAVYA	500
NGEYAGPQDW TSFKEFGEAF SPNYATGEII ITEAFFNAVR DDDIHLTFHY	550
WSGETVEYTL RKNGNYVQGR R	571

Figure 4. Enzyme Activity with Increasing NaCl Concentration

Concentration

HKCEL

O-HKCEL (-1)

Percentage NaCl in CMC Plates

Figure 5. Influence of pH on mHKcel Cellulase Activity

